RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2002; **16**: 2102–2114
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.833

RCM

δ^{13}C and δ^{18}O isotopic composition of $CaCO_3$ measured by continuous flow isotope ratio mass spectrometry: statistical evaluation and verification by application to Devils Hole core DH-11 calcite†‡

Kinga M. Révész* and Jurate M. Landwehr
U.S. Geological Survey, 431 National Center, Reston, VA 20192, USA

Received 1 July 2002; Revised 13 September 2002; Accepted 14 September 2002

A new method was developed to analyze the stable carbon and oxygen isotope ratios of small samples (400 $\pm$ 20 μg) of calcium carbonate. This new method streamlines the classical phosphoric acid/calcium carbonate ($H_3PO_4/CaCO_3$) reaction method by making use of a recently available Thermoquest-Finnigan GasBench II preparation device and a Delta Plus XL continuous flow isotope ratio mass spectrometer. Conditions for which the $H_3PO_4/CaCO_3$ reaction produced reproducible and accurate results with minimal error had to be determined. When the acid/carbonate reaction temperature was kept at 26 °C and the reaction time was between 24 and 54 h, the precision of the carbon and oxygen isotope ratios for pooled samples from three reference standard materials was $\leq$0.1 and $\leq$0.2 per mill or ‰, respectively, although later analysis showed that materials from one specific standard required reaction time between 34 and 54 h for δ^{18}O to achieve this level of precision. Aliquot screening methods were shown to further minimize the total error. The accuracy and precision of the new method were analyzed and confirmed by statistical analysis. The utility of the method was verified by analyzing calcite from Devils Hole, Nevada, for which isotope-ratio values had previously been obtained by the classical method. Devils Hole core DH-11 recently had been re-cut and re-sampled, and isotope-ratio values were obtained using the new method. The results were comparable with those obtained by the classical method with correlation = +0.96 for both isotope ratios. The consistency of the isotopic results is such that an alignment offset could be identified in the re-sampled core material, and two cutting errors that occurred during re-sampling then were confirmed independently. This result indicates that the new method is a viable alternative to the classical reaction method. In particular, the new method requires less sample material permitting finer resolution and allows automation of some processes resulting in considerable time savings. Published in 2002 by John Wiley & Sons, Ltd.

Calcium carbonate or calcite ($CaCO_3$) can be analyzed for the stable isotopes of carbon-13 and oxygen-18 to determine the ratios of the rare (usually the heavy) to the more common (usually the light) isotopes. The classical method used to obtain carbon and oxygen isotope ratios in calcite[1] is labor intensive and requires relatively large sample sizes (10–20 mg). Samples are loaded in a Y-shaped vessel with 100% phosphoric acid (2 mL) in one branch and the carbonate sample in the other. The vessel is evacuated on a vacuum line and placed in a constant temperature bath (25 $\pm$ 0.1 °C). When the temperature stabilizes, each vessel is oriented in such a way as to have the acid flow and react with the carbonate sample following the equation:

$$CaCO_{3(s)} + H_3PO_{4(l)} \rightarrow CaHPO_{4(s)} + H_2O_{(l,g)} + CO_{2(g)} \quad (1)$$

The reaction of acid with calcite produces solid calcium hydrogen phosphate, liquid water, and two gases, water vapor and carbon dioxide. First, both gases are frozen in liquid nitrogen. The frozen gases then are exposed to a dry-ice slush, and the carbon dioxide sublimates while the water stays frozen. After two iterations of melting and then freezing of the gases, it is possible to remove the water vapor from the carbon dioxide. At this point, the carbon dioxide can be analyzed by dual-inlet isotope ratio mass spectrometry (DI-IRMS).

A mass spectrometer is used to determine the ratio (R) of the heavy isotope to the light isotope in a sample. The δ value or proportional difference from a standard is used to report stable isotope abundances and variations. Carbon and

**Correspondence to*: K. M. Révész, U.S. Geological Survey, 431 National Center, Reston, VA 20192, USA.
E-mail: krevesz@usgs.gov
†Use of the brand, firm or trade names in this report is for identification purposes only and does not constitute endorsement by the U.S. Geological Survey.

oxygen isotope data are reported as differences in parts per thousand (per mill or ‰) from their respective reference materials. The δ value is defined as:

$$\delta_X = \left(\frac{R_X - R_{std}}{R_{std}}\right)10^3, \tag{2}$$

where $R_X = (C^{13}/C^{12})_X$ or $(O^{18}/O^{16})_X$ for the sample X, and R_{std} is the corresponding stable isotope ratio in the reference standard.[2] The δ values for the carbon isotope ratio or $\delta^{13}C$ are reported relative to Vienna Peedee Belemnite [VPDB] is defined by $\delta^{13}C_{NBS19/VPDB} = +1.95‰$.[3] The δ values for the oxygen isotope ratio or $\delta^{18}O$ are reported relative to Vienna Standard Mean Ocean Water [VSMOW; $\delta^{18}O_{VSMOW} = 0‰$] on a normalized scale using Standard Light Antarctic Precipitation [SLAP, $\delta^{18}O_{SLAP/VSMOW} = -55.5‰$[4].

The classical preparation was streamlined to make use of robotic technologies and a more sensitive detection method referred to as continuous flow isotope ratio mass spectrometry (CF-IRMS). This new method uses 400 µg of calcite or 2–4% of the sample required in the classical method and only 10% of the acid previously required. To be viable, this new method should provide results that are similar in accuracy and precision to those of the classical method. The purpose of this report is to describe the development and application of this new method.

MATERIALS AND METHODS

The new method makes use of a continuous flow isotope ratio mass spectrometer, the Thermoquest-Finnigan Delta Plus XL. Attached to this mass spectrometer is a preparation device (Thermoquest GasBench II) with a robotic sampling arm (CTC Combi-PAL) by which the sample is ultimately sent to the mass spectrometer. The calcium carbonate samples must first be properly prepared. The sample initially was dried in an oven at 90 °C overnight to prevent any moisture from reacting with carbon dioxide and exchanging an oxygen atom. This drying needed to be done only once, providing that the dried sample was kept in a tightly sealed container when not in use. The sample vessels, 12.5 × 100 mm borosilicate glass (produced by Wheaton), also were dried in an oven at 90 °C for at least 24 h. Once the vessels were removed from the oven, they were capped immediately to keep out moisture. The glass vessels were washed before reuse. Each set of vessels was rinsed eight times with tap water and once with de-ionized (DI) water. An ultrasonic bath was filled with DI water and all of the vessels were submerged. The vessels were cleaned in the ultrasonic bath for 30 min, during which time the water was changed three times, and than removed, emptied and dried as described above.

Each dried sample was weighed on a microbalance in an aluminum boat with a target weight of 400 ± 20 µg. Each sample was transferred quantitatively to a clean and dried sample vessel and capped with a rubber septum (Labco Ltd., pierceable rubber wad, order code VC309). The rubber septum retains an airtight seal after being punctured with a needle. A sample set, consisting of up to 94 vessels containing calcium carbonate, was loaded into the GasBench II autosampler. Each field sample was analyzed at least twice. Vessels containing one of three isotopically different reference materials were interspersed among the samples. No less than one set of reference materials for every eight unknowns was analyzed with weights in the same range as that of the samples.

After the set of samples and reference materials had been assembled, vessels were loaded into the GasBench II tray. The Finnigan Isotope Data acquisition software (ISODAT 7.2) controls the GasBench II preparation device including the CF-IR mass spectrometer. Vessels containing acid were also added to the tray. While carbonate and acid were in the GasBench II, the tray was kept at a constant 26.0 ± 0.1 °C. In this way the acid temperature was identical to that of the sample before it was added. The GasBench II then automatically and individually flushed the samples with helium using a needle to inject, displace and replace the air contained above the samples. Helium is the carrier gas for CF-IRMS; it is inert and reacts with neither the sample nor the mass spectrometer. Helium has a common mass (4), which substantially is different than that of CO_2 (44).

After the flushing process was complete, acid was added to the calcium carbonate. To prevent any water oxygen atoms from exchanging with the carbon dioxide, only 100% phosphoric acid (1.906 g/mL) was used. A gastight syringe was used to manually transfer 0.1 mL of acid into each sample vessel. Care was taken to keep the septum acid free. The samples were left to react with the acid for 24 h and the resulting gases were analyzed automatically within 54 h of the initial acid injection. The "method" in ISODAT was defined such that each individual sample had a sampling set of seven gas aliquots bracketed at both ends by three reference gas injections pulses (Fig. 1).

Subsequently, the data was exported from ISODAT to a

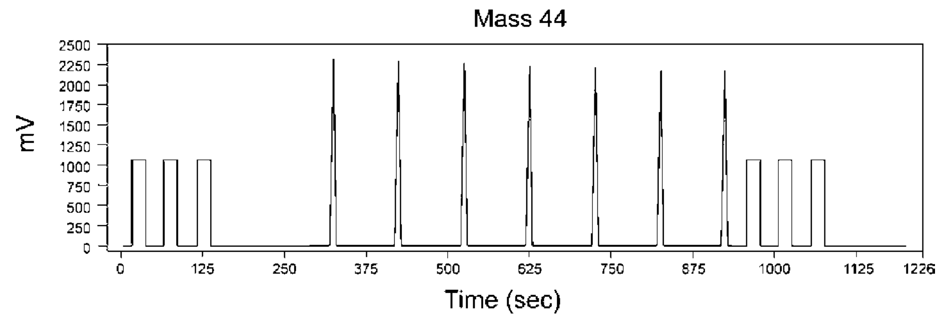

Figure 1. Typical chromatogram of analyses; each individual sample had a sampling set of seven gas aliquots bracketed at both ends by three reference gas injections pulses.

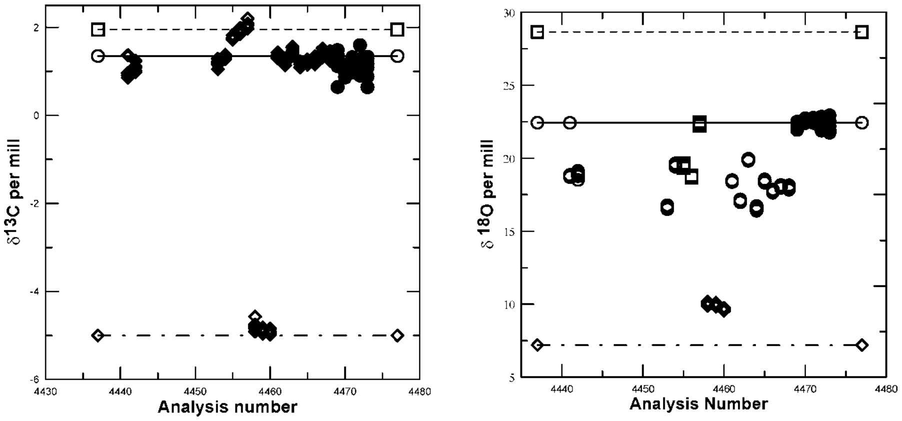

Figure 2. Results of analyzing for $\delta^{13}C$ and $\delta^{18}O$ isotopes of standard reference materials using GasBench II at 65 °C and two different reaction times (overnight shown with open symbols and 1.5 h shown with filled symbols). One analysis number represents seven gas aliquots of a sample. Diamonds represent NBS-18 reference standard; squares are NBS-19, and circles are USGS working standard material; dashed line with dot, dash, and solid lines represent published value of the reference standard, respectively.

laboratory information management system (LIMS),[5] where the final sample values were computed. For each sample, the LIMS computed the δ_X value relative to a working standard, ($\delta^{13}C$ or $\delta^{18}O$ relative to reference gas working standard, Eqn. (2)), by defining the R_{std} value as the average of the six independently computed reference gas ratios that bracketed each sample set. A correction was applied to δ_X to normalize the data relative to VPDB ($\delta^{13}C$) and relative to VSMOW ($\delta^{18}O$). This was computed as the linear fit between the analytic results for the three reference standard materials and their known reference values relative to VPDB and VSMOW, respectively. If the standard deviation among the seven computed δ values of an analysis was larger than the accepted limit (0.1 and 0.2‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively), the analysis was rejected. Similarly, if the difference between the averaged values computed for the two sample aliquots was not within the accepted limits, then the sample was re-analyzed. When sample results were in the correct range, the isotopic ratio was computed on the basis of the 14 gas aliquot results, and the average ratio will have a standard deviation of less than 0.03 and 0.06‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively.

The reaction conditions ultimately chosen after a series of experiments were to maintain the acid reaction at a constant temperature of 26 °C and for a minimum duration of 24 h but a maximum duration less than 54 h. Originally, the samples were reacted at 65 °C, approximately the temperature suggested by the equipment manufacturer. At that temperature, reacted overnight, the carbon isotope ratios for the reference materials were reproducible and accurate, but the oxygen isotope ratios were neither accurate (approximate difference of +3‰; −8‰; −4‰ from reference values of NBS-18; NBS-19 and USGS working standard, respectively) nor reproducible (standard deviation was 1.88‰ among sample runs) (Fig. 2).

The question is whether hot acid reaction for a shorter time would produce acceptable values. The equipment available did not allow this set-up to be automatically configured; therefore, the acid injection procedure was performed manually in the proper timely manner. Shortening the reaction time to 1.5 h improved the reproducibility and accuracy of the oxygen isotope ratios obtained for the working standard material (Fig. 2), but the standard deviation of the seven gas aliquots within an analysis for both isotopes were only marginally acceptable (0.21 and 0.27‰ for $\delta^{13}C$ and $\delta^{18}O$, respectively). This combination of time and temperature has potential if proper equipment is available (e.g. two-arm robot system) and the difficulty of handling high-temperature water vapor (e.g. clogging capillary tubes) can be overcome.

Using a lower reaction temperature (R. Yam, private communication, 2000) and a minimum 24-h reaction time allowed reproducible isotope standards within acceptable range for both, carbon and oxygen (Fig. 3). Because the reaction time affected the oxygen isotope ratios, but not the carbon isotope ratios, it suggests that an exchange reaction for the oxygen isotope occurs during the overnight acid reaction period. The shortened reaction time of 1.5 h at 65 °C gave acceptable, albeit imprecise, results, suggesting that this secondary reaction must have a kinetic component. Therefore, lowering the reaction temperature should inhibit or slow down this secondary reaction. It is important to note that all samples were analyzed within 54 h of acid injection. (Indeed, all but four were analyzed within 47 h.) Because of the kinetic component of the reaction, the method presented in this work should be considered to have an upper boundary of 54 h allowed for reaction time.

Analysis of reference standards

The three reference standard samples were interspersed among field samples that were analyzed over a 5-month period under the identified experimental conditions of 26 °C temperature and overnight (≥24 h) reaction time with acid before analysis. All results of the reference standards were collected into a single dataset, for further analysis, in order to determine the capability of the new method to provide long-

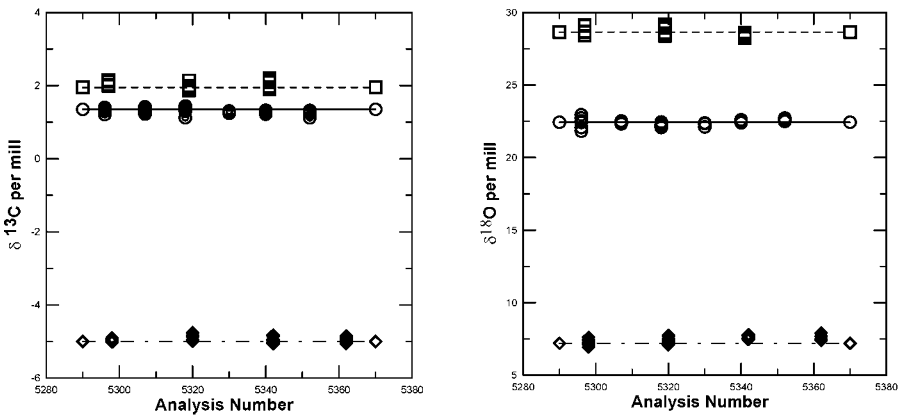

Figure 3. Results of analyzing for $\delta^{13}C$ and $\delta^{18}O$ isotopes of standard reference materials using GasBench II at 26°C and between 24–54 h reaction time. One analysis number represents seven gas aliquots of a sample. Diamonds represent NBS-18 reference standard, squares are NBS-19, and circles are USGS working standard; dashed line with dot, dash, and solid lines represent published value of the reference standard materials, respectively.

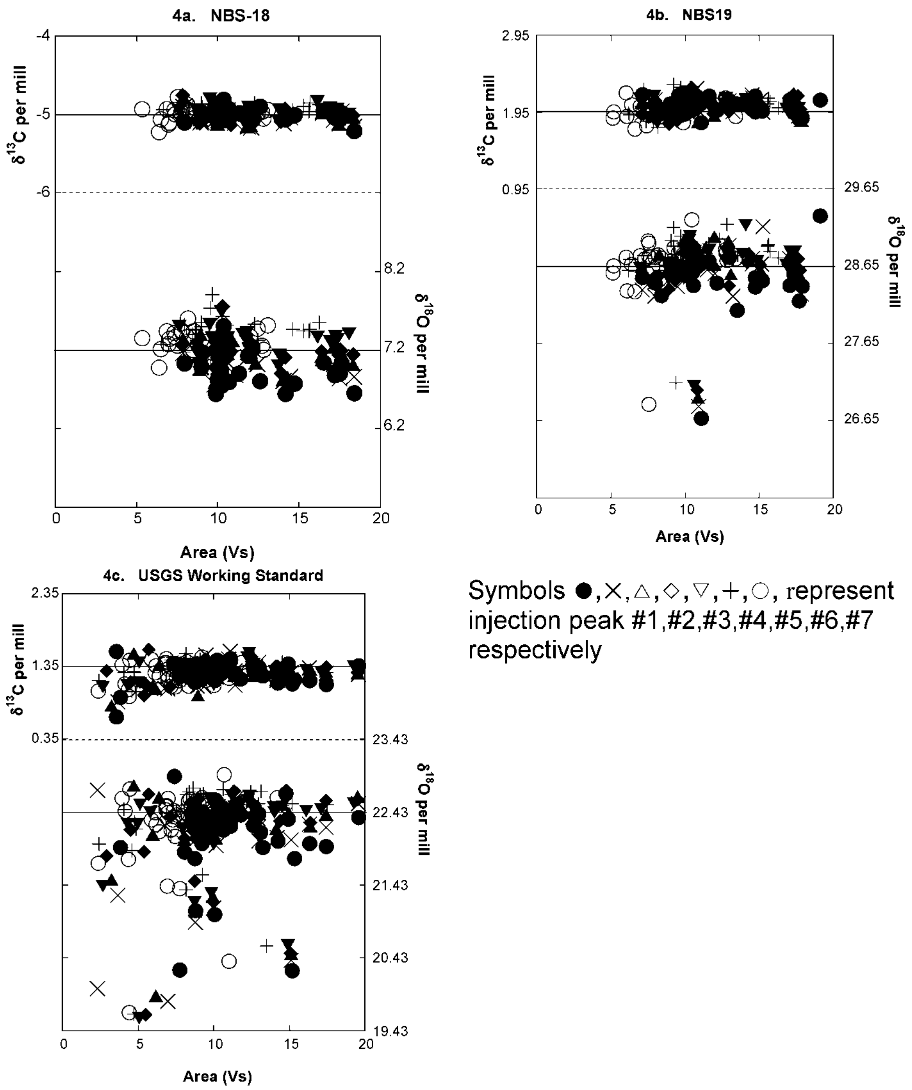

Figure 4. Isotope values for $\delta^{13}C$ and for $\delta^{18}O$ versus area in Volt-seconds for all seven analyses for each sample analyzed from the three reference standards (a = NBS-18; b = NBS-19, c = USGS working standard), totaling 88 samples.

Table 1a. For $\delta^{13}C$ analysis, for all 88 samples from three reference standards run concurrently with field samples, statistics pertaining to bias and pooled standard deviation, among all and pooled analyses and analyses separated by reaction time, and the number of analyses in each category. All units are in‰

Reference category	All analyses	Pooled analyses	Samples analyzed 24–34 h after acid injection	Samples analyzed 34–54 h after acid injection
Average bias				
All samples	−0.01*	−0.01*	−0.01*	−0.005* #
NBS-18	+0.01*	+0.01*	+0.03^R	−0.03* r
USGS working standard	−0.08^R	−0.08^R	−0.10^R	−0.05^R #
NBS-19	+0.08^R	+0.08^R	+0.07^R	+0.09^R #
Standard error of bias				
All samples	0.01	0.01	0.01	0.02
NBS-18	0.01	0.01	0.01	0.02
USGS working standard	0.01	0.01	0.02	0.02
NBS-19	0.01	0.01	0.02	0.03
Standard deviation of bias				
All samples	0.15	0.10	0.11	0.09
NBS-18	0.09	0.05	0.05	0.04
USGS working standard	0.16	0.09	0.09	0.08
NBS-19	0.11	0.07	0.07	0.09
Average pooled standard deviation				
All samples	–	0.09	0.09	0.08
NBS-18	–	0.08	0.08	0.08
USGS working standard	–	0.10	0.12	0.07
NBS-19	–	0.08	0.08	0.10
Number of analyses				
All samples	n = 616	n = 88	n = 61	n = 27
NBS-18	161	23	17	6
USGS working standard	273	39	26	13
NBS-19	182	26	18	8

R: Reject the null hypothesis Ho: average bias = 0 (t-test), at $p \leq 0.05$ level.
*: Cannot reject null hypothesis that average bias is 0.
r: Reject the null hypothesis Ho: average bias (samples with time ≤34 h) = average bias (samples with time >34 h), at $p \leq 0.05$ level.
#: Cannot reject null hypothesis of equality of average bias.

term accurate and precise results. The three reference standards used in this work, NBS-18, USGS working standard and NBS-19, have different isotopic signatures; ordered by increasing values of δ^{13}C and δ^{18}O. NBS-18 with $\delta^{13}C_{VPDB} = -5$‰ and $\delta^{18}O_{VSMOW} = 7.2$‰, the USGS working standard with $\delta^{13}C_{VPDB} = 1.35$‰ and $\delta^{18}O_{VSMOW} = 22.43$‰, and NBS-19 with $\delta^{13}C_{VPDB} = 1.95$‰ and $\delta^{18}O_{VSMOW} = 28.65$‰. Results were inspected graphically (using Grapher version 3 and KaleidaGraph version 3.5 software), and subjected to formal statistical analysis (using Minitab version 10xtra software as well as simple statistics from KaleidaGraph version 3.5 software) to assess bias, precision, total error, effect of sample amount, homogeneity of samples, as well as the effect of the duration of the reaction time from the time the acid was added to a sample up to the time when the sample was analyzed in the mass spectrometer.

A total of 88 samples were analyzed for the three standards: 23 of NBS-18, 39 of the USGS working standard and 26 of NBS-19. Samples amounts were weighed carefully to be ~0.40 mg; the average of the 88 samples was 0.42 ± 0.02 mg, ranging from 0.37 to 0.48 mg. The reaction time was calculated from lab records and noted along with the sample results. Since the analytical procedure for each sample includes seven gas aliquots to the mass spectrometer, a total of 616 = (7 × 88) analyses were made for the three reference standards, with 161 for NBS-18, 276 for the USGS working standard, and 182 for NBS-19.

The isotope ratio mass spectrometer produces voltage-time (Volts-seconds; Vs) peaks for each gas aliquot, where the integrated peak area at the first sample injection is a function of the amount of sample material. The cumulative peak areas produced by the subsequent six other injections diminish in size (Figs 1 and 4). However, the area of the first injection peak is not simply a linear function of the amount of sample material: the linear correlation coefficient between the amount of sample and the first peak was only 0.22, suggesting that some factor in addition to any error in weighing confounded this relationship. The estimates of δ^{13}C and δ^{18}O for each of the seven injections of all 88 analyses by the three reference standards plotted as a function of the cumulative spectrometric peak area for the injection, in Vs units is shown in Fig. 4. The area ranges predominantly from ~20 to ~5 Vs (maximum/minimum ratio = 4) which indicates that the linearity of the mass spectrometer was acceptable and the experimental conditions (ISODAT method, Process File, He flow rate, needle size[6]) were set correctly. In addition, regardless of the initial peak area value, and that of the six subsequent peaks, their minimum value was constrained, suggesting that even a potentially broad range in the sample amount (0.1–0.4 mg)

Table 1b. For $\delta^{18}O$ analysis, for all 88 samples from three reference standards run concurrently with field samples, statistics pertaining to bias and pooled standard deviation, among all and pooled analyses and analyses separated by reaction time, and the number of analyses in each category. All units are in‰

Reference category	All analyses	Pooled analyses	Samples analyzed 24–34 h after acid injection	Samples analyzed 34–54 h after acid injection
Average bias				
All samples	-0.11^{R}	-0.11^{R}	-0.16^{R}	$-0.02^{*\#}$
NBS-18	+0.004*	+0.004*	−0.004*	$+0.03^{*\#}$
USGS working standard	-0.24^{R}	-0.24^{R}	-0.35^{R}	-0.02^{*r}
NBS-19	−0.02*	−0.02*	−0.02*	$-0.04^{*\#}$
Standard error of bias				
All samples	0.02	0.05	0.07	0.02
NBS-18	0.02	0.03	0.04	0.04
USGS working standard	0.04	0.09	0.13	0.04
NBS-19	0.03	0.07	0.10	0.04
Standard deviation of bias				
All samples	0.49	0.45	0.53	0.12
NBS-18	0.24	0.14	0.16	0.10
USGS working standard	0.62	0.57	0.67	0.13
NBS-19	0.40	0.36	0.43	0.11
Average pooled standard deviation				
All samples	–	0.19	0.21	0.14
NBS-18	–	0.20	0.22	0.16
USGS working standard	–	0.20	0.23	0.13
NBS-19	–	0.17	0.18	0.15
Number of analyses				
All samples	n = 616	n = 88	n = 61	n = 27
NBS-18	161	23	17	6
USGS working standard	273	39	26	13
NBS-19	182	26	18	8

R: bias = 0 (t-test), at $p \leq 0.05$ level.
*: Cannot reject null hypothesis that average bias is 0.
r: Reject the null hypothesis Ho: average bias (samples with time $\leq$34 h) = average bias (samples with time between >34 h), at $p \leq 0.05$, level.
#: Cannot reject null hypothesis of equality of average bias.

could produce accurate and reproducible results. Figure 4 demonstrates that the results for the USGS working standard (Fig. 4(c)) were more variable and negatively biased than for either NBS-18 or NBS-19 (Figs 4(a) and 4(b), respectively), even though the isotopic signature of this material is bracketed by the other two. This may reflect the differing mineral composition or grain size of the material.

In order to permit comparisons between reference standards, analytic results were expressed in terms of bias, i.e. the difference between the analytic isotopic estimate and the known isotopic reference standard value. Table 1 provides a statistical summary of results for the 88 samples, with results for $\delta^{13}C$ summarized in Table 1(a), and for $\delta^{18}O$ in Table 1(b). The average bias, the standard deviation of the averaged bias which is called the standard error of the bias and the standard deviation of the bias values are shown when results from all three-reference standards are treated as one set. Results are also summarized for each reference standard set separately, because an analysis of variance rejected ($p \leq 0.001$) the hypothesis of equal average bias among analyses categorized by reference standard. Also, individual analyses were pooled by their sample sets of seven injections, and a bias and standard deviation for these respective sets computed. In addition to the average bias, the average standard deviation for the pooled samples is shown because it is the standard deviation of the pooled set that determines whether or not an aliquot is accepted. Finally, it was seen that the correlation between the amount of sample material and the area of the first injection peak increased from 0.22 to ~0.50 in the case of analyses run with a reaction time between 34–54 h, suggesting some stabilizing factor with longer reaction time. Consequently, the pooled sets were separated into categories by reaction time, from 24–34 h and from 34–54 h, to determine what, if any, effect is obtained with longer reaction times.

It can be seen that the estimate of $\delta^{13}C$ appeared to be unbiased (confirmed by t-test, with $p < 0.05$) over the set of all analyses and all pooled analyses, but the results differed when examined by reference standard (Table 1(a)). The results for NBS-18 are unbiased, the results for the USGS working standard negatively biased, and the results for NBS-19 positively biased; however, the absolute value of the average bias is <0.1‰. The difference in bias between samples with reaction times of 24–34 h and those with reaction time 34–54 h was neither large nor significant (confirmed by F-test using $p \leq 0.05$), except for NBS-18 for which the average bias went from a small positive value to a small negative value but one that was indistinguishable from 0. As expected, pooling reduced the standard deviation of the set, although the standard error of the bias remained at

Table 2a. For $\delta^{13}C$ analysis, for the 71 samples from all three reference standards that abide by screening rules applied to field samples, statistics pertaining to bias and pooled standard deviation, among all and pooled analyses and analyses separated by reaction time, and the number of analyses in each category. All units are in‰

Reference category	All analyses	Pooled samples	Samples analyzed 24–34 h after acid injection	Samples analyzed 34–54 h after acid injection
Average bias				
All samples	0.001*	0.001*	0.01*	−0.005* #
NBS-18	0.01*	0.01*	0.03R	−0.03* r
USGS working standard	−0.06R	−0.06R	−0.07R	−0.05R #
NBS-19	0.08R	0.08R	0.08*	0.09R #
	R	R	R	R
Standard error of bias				
All samples	0.01	0.01	0.01	0.02
NBS-18	0.01	0.01	0.01	0.02
USGS working standard	0.01	0.01	0.02	0.02
NBS-19	0.01	0.02	0.02	0.03
Standard deviation of bias				
All samples	0.12	0.09	0.09	0.09
NBS-18	0.09	0.06	0.05	0.04
USGS working standard	0.10	0.07	0.07	0.08
NBS-19	0.11	0.07	0.06	0.09
Average pooled standard deviation				
All samples	–	0.08	0.07	0.08
NBS-18	–	0.07	0.07	0.08
USGS working standard	–	0.07	0.08	0.07
NBS-19	–	0.08	0.07	0.10
Number of analyses				
All Samples	n = 497	n = 71	n = 44	n = 27
NBS-18	140	20	14	6
USGS Working Standard	210	30	17	13
NBS-19	147	21	13	8

R: Reject the null hypothesis Ho: average bias = 0 (t-test), at $p \leq 0.05$ level.
*: Cannot reject null hypothesis that average bias is 0.
r: Reject the null hypothesis Ho: average bias (samples with time $\leq$34 h) = average bias (samples with time between >34 h), at $p \leq 0.05$, level.
#: Cannot reject null hypothesis of equality of average bias.
R: Reject hypothesis of equal means among reference standards ($p \leq 0.05$).

0.01‰. The standard deviation of the bias is $\leq$0.1‰ for each reference category. Thus, for δ^{13}C, the analyses precisely and accurately reproduce the reference standard values within the desired tolerance ($\leq$0.1‰).

The results for δ^{18}O analyses are summarized in Table 1(b). It is seen that the average bias was not zero (rejected by t-test, at $p < 0.05$ level) for either the set of all analyses or the set of pooled analyses. This is because, although the results for both NBS-18 and NBS-19 are unbiased, those for the USGS working standard indicate a negative bias that was larger than the desired tolerance of 0.2‰. However, when samples were categorized by reaction time, those run with a reaction time 24–34 h were significantly negatively biased but those with reaction time 34–54 h were unbiased. The standard error and the standard deviation were larger for the δ^{18}O analyses than for δ^{13}C. Furthermore, for both NBS-19 and the USGS working standard, the standard deviations were greater than the desired tolerance of 0.2‰ when considered overall samples, but diminished to less than 0.2‰ for samples with reaction time between 34–54 h.

Results for all available analyses, without application of the additional screening rules applied to field sample aliquots described above, are summarized in Table 1. We now consider how these results would change when the more rigorous screening rules that are used to determine aliquot acceptability are applied. In examining the data, in one analysis set (1% of 88) it was found that the integrated peak area of aliquot-7 was larger than aliquot-1 for the sample gas, possibly indicating incorrect sample preparation; consequently, that sample was discarded. According to the rules for examining aliquots stated above, if the standard deviation among the seven injection results was >0.1‰ for δ^{13}C or >0.2‰ for δ^{18}O, the sample was not used. It was found that 14 (16%) of the remaining 87 samples failed with respect to this rule of which 3 (3%) failed just with respect to δ^{13}C, 10 (11%) failed just with respect δ^{18}O, and 1 (1%) failed with respect to both isotopes. In addition, two pooled samples (2%) were found to have an average δ^{18}O estimate differing from any other analysis for their respective standards by ~1.5 and ~0.7‰, respectively, which violates the $\leq$0.2‰ screening rule for aliquots stated above. These samples were not considered further. All pooled samples had an average δ^{13}C value within $\leq$0.1‰ and thus satisfied the screening rule adapted for aliquots as described above. Of the 17 discarded samples, 3 (13% of the initial 23) were for reference standard NBS-18, 9 (23% of 39) for the USGS working standard and 5 (19% of 26) for reference standard NBS-19. It

Table 2b. For $\delta^{18}O$ analysis, for the 71 samples from all three reference standards that abide by the screening rules applied to field samples, statistics pertaining to bias and pooled standard deviation, among all and pooled analyses and analyses separated by reaction time, and the number of analyses in each category. All units are in‰

Reference category	All analyses	Pooled samples	Samples analyzed 24–34 h after acid injection	Samples analyzed 34–54 h after acid injection
Average bias				
All samples	-0.04^{R}	−0.04*	−0.05*	$-0.02^{*\,\#}$
NBS-18	0.01*	0.01*	0.002*	$0.03^{*\,\#}$
USGS working standard	-0.12^{R}	-0.12^{R}	-0.19^{R}	$-0.02^{*\,\#}$
NBS-19	0.03*	0.03*	0.07*	$-0.04^{*\,r}$
	R	R	R	E
Standard error of bias				
All samples	0.01	0.03	0.04	0.02
NBS-18	0.02	0.03	0.05	0.04
USGS working standard	0.02	0.06	0.09	0.04
NBS-19	0.01	0.02	0.03	0.04
Standard deviation of bias				
All samples	0.28	0.23	0.28	0.12
NBS-18	0.23	0.15	0.17	0.10
USGS working standard	0.34	0.31	0.39	0.13
NBS-19	0.18	0.11	0.10	0.11
Average pooled standard deviation				
All samples	---	0.16	0.17	0.14
NBS-18	---	0.18	0.20	0.16
USGS working standard	---	0.15	0.16	0.12
NBS-19	---	0.15	0.15	0.15
Number of analyses				
All samples	n = 497	n = 71	n = 44	n = 27
NBS-18	140	20	14	6
USGS working standard	210	30	17	13
NBS-19	147	21	13	8

R: Reject the null hypothesis Ho: average bias = 0 (t-test), at $p \leq 0.05$ level.
*: Cannot reject null hypothesis that average bias is 0.
r: Reject the null hypothesis Ho: average bias (samples with time ≤34 h) = average bias (samples with time between >34h), at $p \leq 0.05$, level.
#: Cannot reject null hypothesis of equality of average bias.
R: Reject hypothesis of equal means among reference standards ($p \leq 0.05$).
E: Cannot reject hypothesis of equal means with one-way analysis of variance.

was noted that all 17 samples that failed the screening rules had a reaction time between 24 and 34 h.

Of the original 88 samples, 71 (>80%) satisfied the aliquot screening rules. A statistical summary of results for the 71 acceptable samples is provided in Table 2, which follows the format of Table 1. The results for $\delta^{13}C$ are summarized in Table 2(a) and are completely consistent with those in Table 1(a), although with smaller average pooled standard deviation. The results for $\delta^{18}O$ are summarized in Table 2(b). The results are unbiased (confirmed by t-test, with $p < 0.05$) for all cases, except for samples from the USGS working standard, but even for this category the bias is within the desired tolerance of 0.2‰. Both the standard error and the standard deviation were larger among the $\delta^{18}O$ analyses than for $\delta^{13}C$, but the standard deviation was also within the desired tolerance of 0.2‰ for NBS-18 and NBS-19 samples, although larger (0.31‰) for the USGS working standard samples. Samples were separated by reaction time ≤34 h and >34 h. Results between the sets of shorter and longer reaction times differed more for samples from the USGS working standard than for either NBS-18 or NBS-19. Samples run with the shorter reaction time were negatively biased at ∼0.2‰ with a standard deviation of ∼0.4‰, whereas those with reaction time >34 h were unbiased (confirmed with t-test) with standard deviation 0.13‰. (Note: we could not reject the hypothesis of equal means for the average bias for the USGS working standard, but this is a function of the large standard deviation associated with the shorter time samples.) Furthermore, an analysis of variance test among the samples analyzed at >34 h could not reject the hypothesis of equality of average bias among reference standards, although it did so among those of ≤34 h reaction time and for the entire pooled set. Thus, for $\delta^{18}O$, when the screening rules are applied, samples from all three reference standards produced unbiased results with minimum standard deviation (<0.2‰), although it appears that the USGS working standard needs more reaction time for the method to perform correctly. However, given the kinetic nature of the reaction, these results cannot be extrapolated beyond 54 h, the maximum analysis time of these experiments.

Table 3 shows the average root mean square error among all of the pooled samples, and when they were separated by reaction time. This statistic is a metric of the total error in an analysis and is defined as the square root of the sum of the squared bias and the estimated variance; effectively, it is the

RCM

Table 3. Average root mean square error (‰) among pooled samples, for all samples and for samples categorized by reference standard and by reaction time, for samples from all three reference standards, for both $\delta^{13}C$ and $\delta^{18}O$ analysis, and the number of samples in each category

Reference category	All samples	Samples analyzed 24–34 h after acid injection	Samples analyzed 34–54 h after acid injection
δ^{13}C			
All samples	0.11	0.11	0.11
NBS-18	0.09	0.09	0.09
USGS working standard	0.11	0.11	0.11
NBS-19	0.13	0.11	0.15
δ^{18}O			
All samples	0.21	0.24	0.17
NBS-18	0.21	0.23	0.17
USGS working standard	0.24	0.30	0.16
NBS-19	0.18	0.18	0.17
Number of samples			
All samples	n = 71	n = 44	n = 27
NBS-18	20	14	6
USGS working standard	30	17	13
NBS-19	21	13	8

radius of a circle whose center is the true value of a reference where one coordinate axis is the bias, and the other is the standard deviation. The total error in the δ^{18}O is about twice as large in absolute value as that in δ^{13}C, and no significant reduction in error accrues from a longer reaction time in the case of δ^{13}C, but there is a reduction for δ^{18}O, especially in the case of the USGS working standard (Table 3). This metric cannot be used in the case of assessing an unknown sample,

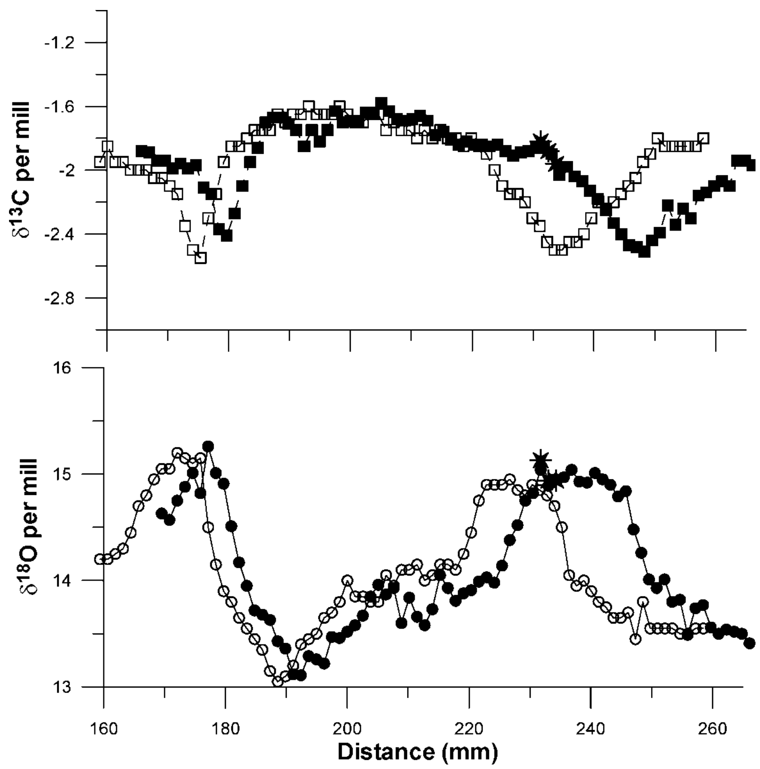

Figure 5. Stable carbon and oxygen isotope ratio profiles obtained by the GasBench II method for the re-sampled DH-11 core material compared with the original DH-11 analysis by the classical method. Filled symbols indicate data profiles of the re-sampled DH-11 core material obtained by the GasBench II method and star symbols indicate analysis of the same core by the classical method; open symbols indicate data profiles from the original DH-11 analysis obtained by the classical method, as reported by Landwehr *et al.*[8]

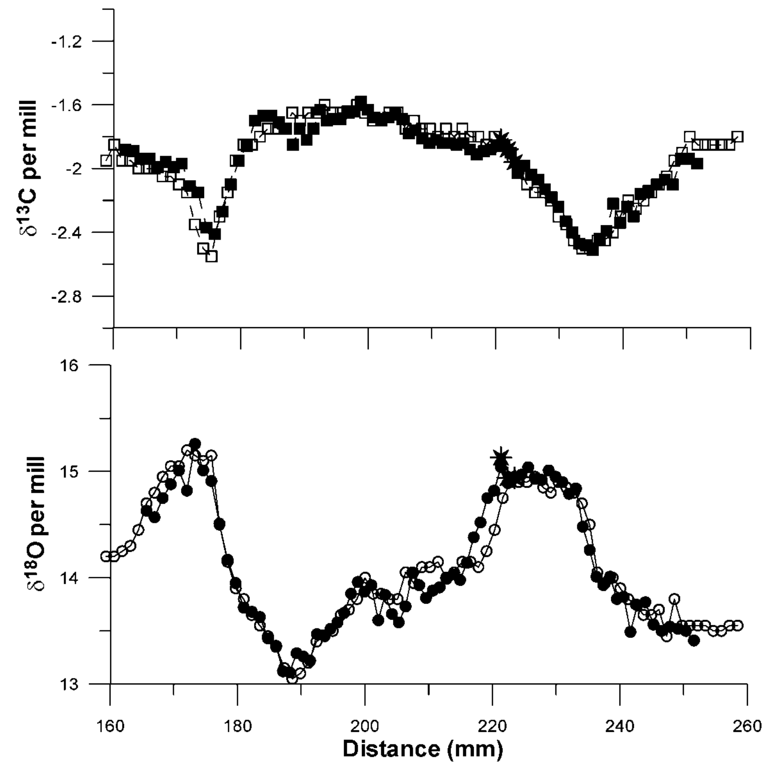

Figure 6. Stable carbon and oxygen isotope ratio profiles obtained by the GasBench II method for the re-sampled DH-11 core material, with appropriate sampling depth correction applied, compared with the original DH-11 analysis by the classical method. Filled symbols indicate data profiles of the re-sampled DH-11 core material obtained by the GasBench II method and star symbols indicate analysis of the same core by the classical method; open symbols indicate data profiles from the original DH-11 analysis obtained by the classical method, as reported by Landwehr *et al.*[8] The linear correlation between the isotope values generated by the classical and the new method is >0.96 for both isotopes.

but is useful for screening results in the case of known reference standards, or for inter-laboratory comparisons.

Testing of method by application to Devils Hole calcite

Calcite from Devils Hole, Nevada, was used to test this new method. Devils Hole is a tectonic cave formed in the discharge zone of a regional carbonate-rock aquifer in south-central Nevada (36 °N, 116 °W). Dense vein calcite has precipitated from the ground water onto the walls of this sub-aqueous cavern.[7] Devils Hole core DH-11 is a 36-cm long core taken from the wall of the cave at about 30 m below the water table; it contains an approximately 500 000-year-old continuous record of the paleoclimate.[8] The core originally was sampled along its length at approximately 1.27-mm intervals by milling and analyzed for stable isotopic composition by the classical method; it was uranium-series dated using thermal ionization mass spectrometry (TIMS).[9] In 1998, a new slab was cut from DH-11 for trace-element determination. We analyzed the C and O stable isotope ratios in the new slab samples taken again in 1.27-mm intervals at distances from 165.7 to 266.0 mm from the free (outer) face of the specimen. The intention was to use the $\delta^{13}C$ and $\delta^{18}O$ analyses of the 1998 sample to date the isotopic time series of the new slab by matching it with the time series from the original slab (analyzed by the classical method) of DH-11.

Calcite re-sampled from core DH-11 was analyzed by the new method. The analytical results, compiled into a data table organized by the reported re-sampling depth of each sample, are given in Appendix A. The stable isotope data plotted with the re-sampled depth is shown in Fig. 5. An inverse relationship results between the oxygen and carbon isotope ratio data, consistent with the pattern reported by Coplen *et al.*[10]

For a check of method consistency, three samples of the re-sampled material (at approximately 232, 233, and 234 mm) also were analyzed using the classical method. These data are shown in Fig. 5 as stars and listed at the bottom of the table in Appendix A. All results differ by less than 0.1‰ and this corroborates the consistency of the two methods.

When the series from the re-sampled material (filled symbols in Fig. 5) was compared with the original data (open symbols), two locations from which offsets increased nonlinearly with depth were identified (Fig. 5). This observation was confirmed to correspond with periodic re-positioning of the new slab during milling when it was observed to be slipping in the vise (I. J. Winograd, private communication, 2001). A mathematical correction to the recorded cutting depths was applied to accommodate these re-sampling conditions (Fig. 6).

When the results of the new method, with corrected sampling depths, are compared with the classical method (Fig. 6), it can be seen that the new method reproduced the isotopic trace produced by the classical method. This is demonstrated by a linear correlation between isotope values of approximately 0.96 for both isotopes, after correcting for an alignment offset. Hence, the new method can be used as an alternative to the classical method. In the case of the DH-11 sample, both methods allow one to stratigrafically align cores by matching stable isotope patterns, a useful capability in paleoclimate studies; however, because the new method requires less sample material, it permits finer resolution over the length of the core, thus finer temporal resolution of the material.

In addition, this work demonstrates the utility of the method for making stratigraphic connections between cores by matching stable isotope patterns, e.g. allowing one to age-date new core material by reference to previously dated material.

CONCLUSIONS

A new method was developed to analyze small samples (approximately 400 μg) of calcium carbonate (calcite) for the stable isotope ratios of carbon and oxygen. The new method streamlines the classical $H_3PO_4/CaCO_3$ reaction method by making use of a Thermoquest-Finnigan GasBench II preparation device and Delta Plus XL continuous-flow isotope ratio mass spectrometer. Results from analysis of three different reference materials were evaluated statistically; the method was seen to produce unbiased and precise estimates of $\delta^{13}C$ when the acid reaction time was between 24 and 54 h, and of $\delta^{18}O$ when the reaction time between 34 and 54 h. Aliquot screening methods were shown to further minimize the total error. The method was tested by analyzing calcite from Devils Hole, Nevada, core DH-11, and its utility is confirmed by its capability to reproduce results, thereby allowing one to stratigrafically align cores by matching stable isotope patterns.

Care must be taken to check the precision of the standards, because reruns might be necessary. These additional analyses require time, but overall the automation of the GasBench II method results in considerable time-savings. An additional advantage of the new method is that it requires less sample materials; hence, allowing analysis when sample amount is limited or when finer sampling resolution is needed. These advantages make this method a viable alternative to the classical method.

Acknowledgements

We thank Tyler B. Coplen for his support in all aspects of the project, Isaac J. Winograd for his help in ascertaining the 1998 re-sampling procedure and for many useful discussions, and Jerry Keybl for assistance in this project.

APPENDIX A. Data tabulation

Our Lab ID	Field ID	Resampling depth (in.)	Resampling depth (mm)	Corrected depth (mm)	Isotopes (‰) δ^{13}C	Isotopes (‰) δ^{18}O
Run with new method						
C-10699	DH-11 6.50–6.55	6.525	165.735	161.935	−1.88	–
C-10700	DH-11 6.55–6.60	6.575	167.005	163.205	−1.89	–
C-10701	DH-11 6.60–6.65	6.625	168.275	164.475	−1.94	–
C-10702	DH-11 6.65–6.70	6.675	169.545	165.745	−1.94	14.63
C-10703	DH-11 6.70–7.75	6.725	170.815	167.015	−1.99	14.57
C-10704	DH-11 6.75–6.80	6.775	172.085	168.285	−1.96	14.75
C-10705	DH-11 6.80–8.85	6.825	173.355	169.555	−1.99	14.88
C-10706	DH-11 6.85–6.90	6.875	174.625	170.825	−1.97	15.01
C-10707	DH-11 6.90–6.95	6.925	175.895	172.095	−2.11	14.82
C-10708	DH-11 6.95–7.00	6.975	177.165	173.365	−2.15	15.26
C-10709	DH-11 7.00–7.05	7.025	178.435	174.635	−2.37	15.01
C-10710	DH-11 7.05–7.10	7.075	179.705	175.905	−2.41	14.91
C-10711	DH-11 7.10–7.15	7.125	180.975	177.175	−2.27	14.51
C-10712	DH-11 7.15–7.20	7.175	182.245	178.445	−2.10	14.17
C-10713	DH-11 7.20–7.25	7.225	183.515	179.715	−1.95	13.95
C-10714	DH-11 7.25–7.30	7.275	184.785	180.985	−1.86	13.72
C-10715	DH-11 7.30–7.35	7.325	186.055	182.255	−1.70	13.68
C-10716	DH-11 7.35–7.40	7.375	187.325	183.525	−1.67	13.63
C-10717	DH-11 7.40–7.45	7.425	188.595	184.795	−1.67	13.43
C-10718	DH-11 7.45–7.50	7.475	189.865	186.070	−1.71	13.36
C-10719	DH-11 7.50–7.55	7.525	191.135	187.139	−1.75	13.12
C-10720	DH-11 7.55–7.60	7.575	192.405	188.208	−1.85	13.11
C-10721	DH-11 7.60–7.65	7.625	193.675	189.277	−1.75	13.29
C-10722	DH-11 7.65–7.70	7.675	194.945	190.347	−1.82	13.26
C-10723	DH-11 7.70–7.75	7.725	196.215	191.416	−1.75	13.22
C-10724	DH-11 7.75–7.80	7.775	197.485	192.485	−1.63	13.47
C-10725	DH-11 7.80–7.85	7.825	198.755	193.554	−1.70	13.46
C-10726	DH-11 7.85–7.90	7.875	200.025	194.623	−1.69	13.52
C-10727	DH-11 7.90–7.95	7.925	201.295	195.692	−1.69	13.58
C-10728	DH-11 7.95–8.00	7.975	202.565	196.761	−1.64	13.67
C-10729	DH-11 8.00–8.05	8.025	203.835	197.830	−1.64	13.85
C-10730	DH-11 8.05–8.10	8.075	205.105	198.900	−1.58	13.96
C-10731	DH-11 8.10–8.15	8.125	206.375	199.969	−1.63	13.87
C-10732	DH-11 8.15–8.20	8.175	207.645	201.038	−1.68	13.93
C-10733	DH-11 8.20–8.25	8.225	208.915	202.107	−1.69	13.60
C-10734	DH-11 8.25–8.30	8.275	210.185	203.176	−1.68	13.84
C-10735	DH-11 8.30–8.35	8.325	211.455	204.245	−1.66	13.66
C-10736	DH-11 8.35–8.40	8.375	212.725	205.314	−1.69	13.58
C-10737	DH-11 8.40–8.45	8.425	213.995	206.383	−1.78	13.73
C-10738	DH-11 8.45–8.50	8.475	215.265	207.453	−1.76	14.05
C-10739	DH-11 8.50–8.55	8.525	216.535	208.522	−1.81	13.93
C-10740	DH-11 8.55–8.60	8.575	217.805	209.591	−1.84	13.81
C-10741	DH-11 8.60–8.65	8.625	219.075	210.660	−1.82	13.88
C-10742	DH-11 8.65–8.70	8.675	220.345	211.729	−1.84	13.91
C-10743	DH-11 8.70–8.75	8.725	221.615	212.798	−1.84	13.99
C-10744	DH-11 8.75–8.80	8.775	222.885	213.867	−1.85	14.03
C-10745	DH-11 8.80–8.85	8.825	224.155	214.937	−1.84	13.98
C-10746	DH-11 8.85–8.90	8.875	225.425	216.006	−1.88	14.14
C-10747	DH-11 8.90–8.95	8.925	226.695	217.075	−1.91	14.38
C-10748	DH-11 8.95–9.00	8.975	227.965	218.144	−1.89	14.52
C-10476	DH-11 9.00–9.05	9.025	229.235	219.213	−1.88	14.75
C-10477	DH-11 9.05–9.10	9.075	230.505	220.282	−1.86	14.82
C-10478	DH-11 9.10–9.15	9.125	231.775	221.351	−1.86	15.04
C-10479	DH-11 9.15–9.20	9.175	233.045	222.420	−1.90	14.89
C-10480	DH-11 9.20–9.25	9.225	234.315	223.490	−2.03	14.95
C-10481	DH-11 9.25–9.30	9.275	235.585	224.559	−1.98	14.97
C-10482	DH-11 9.30–9.35	9.325	236.855	225.628	−2.04	15.04
C-10483	DH-11 9.35–9.40	9.375	238.125	226.697	−2.07	14.93
C-10484	DH-11 9.40–9.45	9.425	239.395	227.766	−2.13	14.92
C-10485	DH-11 9.45–9.50	9.475	240.665	228.835	−2.18	15.01
C-10486	DH-11 9.50–9.55	9.525	241.935	229.904	−2.24	14.95
C-10487	DH-11 9.55–9.60	9.575	243.205	230.973	−2.33	14.90
C-10488	DH-11 9.60–9.65	9.625	244.475	232.043	−2.40	14.79
C-10489	DH-11 9.65–9.70	9.675	245.745	233.112	−2.47	14.84

APPENDIX A. Data tabulation (continued)

Our Lab ID	Field ID	Resampling depth (in.)	Resampling depth (mm)	Corrected depth (mm)	Isotopes (‰) δ^{13}C	Isotopes (‰) δ^{18}O
C-10490	DH-11 9.70–9.75	9.725	247.015	234.181	−2.48	14.48
C-10491	DH-11 9.75–9.80	9.775	248.285	235.250	−2.51	14.26
C-10492	DH-11 9.80–9.85	9.825	249.555	236.319	−2.44	14.01
C-10493	DH-11 9.85–9.90	9.875	250.825	237.388	−2.39	13.93
C-10494	DH-11 9.90–9.95	9.925	252.095	238.457	−2.22	14.01
C-10495	DH-11 9.95–10.00	9.975	253.365	239.527	−2.34	13.80
C-10496	DH-11 10.00–10.05	10.025	254.635	240.596	−2.24	13.82
C-10497	DH-11 10.05–10.10	10.075	255.905	241.665	−2.30	13.49
C-10498	DH-11 10.10–10.15	10.125	257.175	242.734	−2.16	13.74
C-10499	DH-11 10.15–10.20	10.175	258.445	244.000	−2.14	13.77
C-10500	DH-11 10.20–10.25	10.225	259.715	245.270	−2.10	13.56
C-10501	DH-11 10.25–10.30	10.275	260.985	246.540	−2.07	13.50
C-10502	DH-11 10.30–10.35	10.325	262.255	247.810	−2.10	13.54
C-10503	DH-11 10.35–10.40	10.375	263.525	249.080	−1.94	13.52
C-10504	DH-11 10.40–10.45	10.425	264.795	250.350	−1.94	13.50
C-10505	DH-11 10.45–10.50	10.475	266.065	251.620	−1.97	13.41
Rerun with classical method						
C-10478	DH-11 9.10–9.15	9.125	231.775	221.351	−1.82	15.13
C-10479	DH-11 9.15–9.20	9.175	233.045	222.420	−1.88	14.94
C-10480	DH-11 9.20–9.25	9.225	234.315	223.490	−1.96	14.94

REFERENCES

1. McCrea JM. *J. Chem. Phys.* 1950; **18**: 849.
2. Friedman I, O'Neil JR. *U.S. Geological Survey Professional Paper 440-KK*, (6th edn), Fleischer M (ed). U.S. Government Printing Office, Washington, DC, 1977; 12, plus illustrations.
3. Hut G. *Consultants' Group Meeting on Stable Isotope Reference Samples for Geochemical and Hydrological Investigations*, Vienna, 16–18 September, 1985. Report to Director General, International Atomic Energy Agency. 1987; 42.
4. Coplen TB. *Chem. Geol.* 1988; **72**: 293.
5. Coplen TB. *U.S. Geological Survey Open-File Report 00-345*. 2000; 121.
6. Revesz K, Landwehr JM, Kebly J. *U.S. Geological Survey Open-File Report N:01-257*; 2001.
7. Winograd IJ, Landwehr JM, Ludwig KR, Coplen TB, Riggs AC, Szabo BJ, Kolesar PT, Revesz KM. *Science* 1992; **258**: 255.
8. Landwehr JM, Coplen TB, Ludwig KR, Winograd IJ, Riggs AC. *U.S. Geological Survey Open-File Report 97–792*. 1997; 8.
9. Ludwig KR, Simmons KR, Szabo BJ, Winograd IJ, Landwehr JM, Riggs AC, Hoffman RJ. *Science* 1992; **258**: 284.
10. Coplen TB, Winograd IJ, Landwehr JM, Riggs AC. *Science* 1994; **263**: 361.